Progress Report

9502386 Coat protein introgression and virus spread in *Cucurbita pepo* and *C. texana*.

D. Gonsalves
Department of Plant Pathology
Cornell University
Geneva, NY 14456-0462
Phone: 315-787-2334
Email: dg12@nysaes.cornell.edu

1) Evaluate the relative fitness of F1 hybrids of Cucurbita pepo ssp. ovifera var. ovifera (C. pepo) x Cucurbita pepo ssp. ovifera var. texana (C. texana) that contain the viral coat protein genes of cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus II (WMV II) and subsequent backcrosses with C. texana.

2) Under natural conditions, measure the introgression of the coat protein genes of CMV, ZYMV, and WMV II from F1 hybrids of transgenic C. pepo x C. texana to C. texana over several generations

3) Assess the plant to plant spread of a non-aphid transmissible ZYMV strain after its introduction into uniform large-scale fields of transgenic squash that express the coat protein gene of an aphid transmissible WMV II strain.

Results:

Experiments were set up under conditions of heavy and no or very low virus pressures. Under low virus pressure, CP transgenes (and hence virus resistance) to the specific viruses was transferred to C. texana under our field conditions where the F1 transgenic plants of the squash x C. texana cross and nontransgenic C. texana were in close proximity (1-4 meters). Transfer of transgenes to C. texana was apparently by natural pollination mediated by insects (most likely bees) and occurred through two consecutive generations. In contrast, under high virus pressure, transfer of transgenes to C. texana virtually did not occur because the C. texana plants were severely affected by the virus and produced very few flowers.

Our results suggest that C. texana that contain the virus resistant genes will continue to thrive under natural conditions under heavy virus pressure, whereas C. texana that are not resistant will be severely affected. How do our results apply to the potential weediness of C. texana? Observations suggest that C. texana is not a noxious weed. Would it become a noxious weed if it became virus resistant? The important practical factor in the risk assessment, thus, is whether viruses (in particular ZYMV, WMV II, and CMV) are currently limiting the growth (and thus the potential weediness) of C. texana throughout the US. If it is, then introduction of virus resistance to C. texana might be of potential risk. This condition would apply whether the resistant genes came from transgenic plants or from resistant cultivars that obtained their resistant genes from wild relatives of squash. Our research points to the need for further surveys of C. texana to determine if viruses are really limiting their growth in the US.

As expected, we found that back crossing (under control conditions) of the virus resistance cultivar squash to C. texana caused the development of plants that had C. texana-like growth characteristics in addition to being virus resistant. The main impact of this work is that we showed that virus resistant plants with growth habitats of C. texana could develop over several generations of back crossing.

Another concern regarding transgenic plants is related to the area of heteroencapsidation, where transgenic plants expressing the coat protein gene of an aphid transmissible virus may mediate the spread of a non-aphid transmissible isolate of the virus or other unrelated viruses. We investigated this subject with transgenic squash expressing the aphid transmissible strain of WMV II and a ZYMV isolate that was non-aphid transmissible due to a defect in its coat protein gene. Experiments were done under field conditions.

Our results showed that the transgenic squash mediated the transmission of the non-aphid transmissible ZYMV strain to a low percentage (1-2%) of surrounding transgenic or nontransgenic squash. Furthermore, we did not observe further spread of the non-aphid transmissible strain. The non-aphid transmissible isolate did not spread in plots where the virus was exposed to only nontransgenic plants. Taken together, these data suggest that the transgenic plants can mediate the transmission of non-aphid transmissible ZYMV. However, we have no evidence that the transmission rates will cause epidemics.

These aphid transmission experiments confirmed laboratory experiments which showed that transgenic plants expressing coat protein genes could mediate the spread of non-aphid transmissible viruses. Our results suggest that heteroencapsidation will not cause epidemics and thus is not likely to have significant consequences to the environment.

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